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Synthesis of Cationic Dumbbell-shaped Fullerene Nanostructures as Potential Photodynamic Sensitizers

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A design of novel hydrophilic tetracationic dumbbell-shaped [60]fullerene nanostructures was made by balancing the hydrophilicity and hydrophobicity characteristics of the fullerene adduct for their potential application as photodynamic sensitizers in the PDT treatment. A sequential protection-deprotection reaction pathway was applied for the functional differentiation between primary and secondary amine moieties of pentaethylene hexamine. Synthesis of the target molecule involves two key steps of unsymmetrical esterification and amidation of malonic acid and subsequent fullerenation. The synthetic strategy was accomplished using mild reaction conditions in the intermediate molecule preparation and led a moderate overall product yield.

Keywords: Tetracationic fullerene derivatives, dumbbell-shaped nanostructures, photosensitizer, photodynamic therapy, amphiphilic fullerenes

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1 Introduction

An advantage of laser irradiation targeting directly on disease tissues leading to their destruction using the method of photodynamic therapy (PDT) allows the application as an alternative approach to radio- and chemotherapy against malignant cells, in the contrast of minimizing the cytotoxicity on the normal cells in a remote distance from the light beam (1).The FDA approved sensitizers for clinical PDT practices included Photofrin II, an enriched active fraction of hematoporphyrin derivatives (2), and disulfonated aluminum phthalocyanine (AlS_2Pc) (3–5). The main biological function of photoexcited organic chromophore-based sensitizers is to induce severe oxidative damage to the chemical or physical structure of lipids, proteins, and nucleic acids (6), which are associated as major components of the subcellular plasma membrane, mitochondria, cytoplasmic organelles, lysosomes, enzymes, and DNA (7). Accordingly, external oxidative stress-induced structural decomposition or rapid deterioration of biological functions and responses resulted in vascular disruption of the cell and direct tumor cell death (8). In the concept of fundamental photophysical chemistry, excitation of an organic chromophore by photoenergy absorption leads to the injection of one ground-state electron to its excited singlet state. It is followed by relocation of this electron to the corresponding excited triplet state that subsequently relaxes back to the ground state by passing the same energy to molecular triplet oxygen producing singlet oxygen, giving the cell cytotoxicity (9). Other than $^1O_2$, significant involvement of superoxide radicals during the process of the cell death was also reported recently as the plausible cause of the biological mortality (10).

Most of monofunctionalized [60] fullerene derivatives are capable of performing the intermolecular photoenergy or electron transfer (in the presence of biological donor) from excited $^3C_{60}$*-R or $(C_{60})^{-}$·-R, respectively, to O_2 that leads to the production of reactive oxygen species (ROS). The event can be correlated to the photoinduced cytotoxicity partly in connection with the DNA cleavage (11). Early reports on *in vivo* photodynamic effect of hydrophilic fullerene derivatives, such as polyethylene glycol-conjugated C_{60} (PEG–C_{60}) (12)and micelle-like hexa(sulfo-*n*-butyl)[60]fullerenes (FC_4S) (13), were recognized. Absorption of photoenergy in UV to visible wavelengths by the C_{60} cage injects one ground state electron from the HOMO energy level to the excited LUMO energy level even though functionalized fullerenes remain the possession of a low absorption coefficient value in the visible region. Relocation of this electron to the triplet state energy level with subsequent relaxation back to the ground state becomes the origin of photogenerated singlet oxygen(14–16) in a mechanism resembling other organic photosensitizers mentioned above. In addition to $^1O_2$, increasing evidence involving other reactive oxygen species in photoinduced cytotoxicity of biological tissues was also reported by the study of

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rat liver microsome membranes during photosensitization using [60]fullerenol, $C_{60}(OH)_{18}$, as a sensitizer (17). In this report, a generation of highly reactive hydroxyl radicals was proposed in the presence of biological medium. These ROS intermediates, such as $O_2^{-}\cdot$ and ·OH, can be confirmed via spin-trapping *e.s.r.* measurement with a trapping agent of 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) using PVP-solubilized C_{60} and C_{70} molecules as a sensitizer (18).

In this article, we describe the design and synthesis of hydrophilic tetracationic dumbbell-shaped [60]fullerene nanostructures for the potential application of these materials as photodynamic sensitizers in the PDT treatment. Preparation of the target molecule involves a sequential protection-deprotection reaction of pentaethylene hexamine, leading to a linear chain containing multiple quaternary ammonium salts. Surprisingly, the attachment of highly hydrophobic fullerene cage to an incompatible, highly hydrophilic quaternary ammonium chain can be carried out with the proper selection of the size of alkyl moieties, giving a moderate overall reaction product yield.

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2 Experimental

2.1 Materials

Reagents of pentaethylene hexamine, ethyl trifluoroacetate, *n*-butyraldehyde, sodium triacetoxyborohydride, triethylene glycol monomethyl ether, 2,2-dimethyl-1,3-dioxane-4,6-dione, and iodomethane were purchased from Aldrich Chemicals and used without further purification. *N*-hydroxysuccinimide was purchased from Alfa Aesar and all other chemicals were purchased from Acros Ltd. Sodium sulfate was employed as a drying agent. Solvents were routinely distilled.

2.2 Spectroscopic Measurements

Infrared spectra were recorded as KBr pellets on a Nicolet 750 series FT-IR spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on either a Bruker Avance Spectrospin–500 or Bruker AC-250 spectrometer. UV-Vis spectra were recorded on a Hitachi U-3410 UV spectrometer.

2.2.1 *Synthesis of 1,16-di(trifluoroaceto)-pentaethylene-hexamine 1*

To a solution of pentaethylene hexamine (5.0 g, 21.5 mmol) in anhydrous tetrahydrofuran (75 mL), ethyl trifluoroacetate (6.1 g, 43.1 mmol) at 0°C was added slowly over a period of 15 min with stirring. The reaction was allowed to continue for an additional period of 1.0 h at 0°C. The solvent was removed to give pure 1,16-di(trifluoroaceto)-pentaethylenehexamine **1** as light yellow viscous liquid in 98% yield (8.94 g). Spectroscopic data of the compound **1**: FT-IR (KBr) υ_{max} 3297 (w), 2943 (w), 2895 (w), 2824 (w), 1704 (s), 1560 (m), 1241 (vs), 1206 (m), 1144 (m), 802 (m), and 722 (m) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$, ppm) δ 3.41 (t, $J = 4.59$ Hz, 4H), 2.64 (m, 16H), and 1.78 (s, br, 2H).

2.2.2 *Synthesis of Pentaethylene-3,6,9,12-tetra(n-butylamine)-1,14-di(trifluoroacetamide) 2*

A mixture of 1,16-di(trifluoroaceto)-pentaethylenehexamine **1** (5.0 g, 11.78 mmol) and *n*-butyraldehyde (3.74 g, 51.86 mmol) were stirred in anhydrous 1,2-dichloroethane (100 mL) for 30 min at room temperature. The reaction mixture was treated with sodium triacetoxyborohydride (15 g, 70.7 mmol) with stirring for a period of 12 h at room temperature under N_2 atmosphere. It was subsequently quenched by adding aqueous saturated sodium bicarbonate solution. The organic products were extracted by chloroform, dried over Na_2SO_4, and concentrated on a rotary evaporator. The resulting crude product was treated with dil. HCl (2.0 N) and washed by diethyl ether. The aqueous solution was then neutralized by sodium carbonate (5%) and extracted with diethyl ether. The diethyl ether layer was dried over Na_2SO_4 and concentrated on rotavap to afford the product pentaethylene-3,6,9,12-tetra(*n*-butylamine)-1,14-di(trifluoroacetamide) **2** in 95% yield (7.26 g) as yellow viscous liquid. Spectroscopic data of the compound **2**: FT-IR (KBr) υ_{max} 3312 (w), 2957 (m), 2930 (m), 2869 (w), 2815 (w), 1705 (s), 1553 (m), 1462 (m), 1206 (s), 1153 (vs), 756 (m), and 724 (m) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$, ppm) δ 3.38 (m, 6H), 2.56 (m, 24H), 1.38 (m, 8H), 1.28 (m, 8H), and 0.90 (t, $J = 6.95$, 12H).

2.2.3 *Synthesis of 1,14-diamino-pentaethylene-3,6,9,12-tetra(n-butylamine) 3, N_6C_4*

Pentaethylene-3,6,9,12-tetra(*n*-butylamine)-1,14-di(trifluoroacetamide) **2** (7.0 g, 10.78 mmol) was added to a solution of potassium carbonate (7%) in a mixture of MeOH–H_2O (7:3, 100 mL). The mixture was stirred at ambient temperature for a period of 6.0 h. At the end of the reaction, the solvent was evaporated and water (50 mL) was added to the residue, it was then extracted with chloroform (2 × 50 mL). The organic phase was washed further with water (30 mL) and a brine solution (30 mL) in sequence and dried over Na_2SO_4. After solvent evaporation, the product 1,14-diamino pentaethylene-3,6,9,12-tetra(*n*-butylamine) **3**,N_6C_4, was obtained in 85% yield (4.18 g) as yellow liquid. Spectroscopic data of the compound **3**: FT-IR (KBr) υ_{max} 2954 (s), 2930 (s), 2864 (m), 2804 (m), 1461 (s), 1375 (w), 1219 (w), 1085 (w), and 772 (vs) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$, ppm) δ 2.72 (m, 4H), 2.43–2.53 (m, 24H), 1.42 (m, 8H), 1.30 (m, 8H), and 0.91 (t, $J = 7.05$ Hz, 12H).

2.2.4 *Synthesis of Malonic Acid Methoxytriethyleneglycol ester 4, MEG_3*

A mixture of triethylene glycol monomethyl ether (5.0 g, 30.45 mmol) and 2,2-dimethyl-1,3-dioxane-4,6-dione (4.38

g, 30.45 mmol) were heated at 95°C for a period of 6.0 h. The reaction mixture was cooled to room temperature, treated with aqueous sodium carbonate (5%) solution, and washed with diethyl ether. The resulting aqueous layer was subsequently treated with dil. HCl (2.0 N) and extracted with ethyl acetate (50 mL). The ethyl acetate solution was dried over Na_2SO_4 and concentrated on rotavap to give the product malonic acid methoxytriethyleneglycol ester **4**, MEG_3, as a colorless liquid in 95% yield (7.24 g). Spectroscopic data of the compound **4**: FT-IR (KBr) υ_{max} 3056 (w), 2880 (w), 2817 (w), 1728 (vs), 1455 (w), 1394 (m), 1318 (s), 1251 (s), 1134 (vs), 1098 (vs), 1034 (m), 952 (w), 847 (s), and 753 (m) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$, ppm) δ 10.22 (s, br, 1H), 4.31 (t, $J = 4.31$ Hz, 2H), 3.60–3.75 (m, 10H), and 3.36–3.42 (m, 5H).

2.2.5 *Synthesis of 1,14-pentaethylene-3,6,9,12-tetra(n-butylamine)-bis(methoxytriethylene glycol ester malonamide) 5, $(MEG_3)_2N_6C_4$*

A mixture of malonic acid methoxytriethyleneglycol ester **4** (1.0 g, 3.99 mmol), *N*-hydroxysuccinamide (0.45 g, 3.99 mmol), and *N*, *N*'-dicyclohexyl carbodiimide (0.82 g, 3.99 mmol) in anyhydrous tetrahydrofuran (30 mL) were stirred under N_2 atmosphere for a period of 12 h at ambient temperature. The resulting white solids of *N*, *N*'-dicyclohexyl urea were filtered and the filtrate was taken into a second round-bottom flask containing 1,14-diaminopentaethylene-3,6,9,12-tetra(*n*-butylamine) **3** (0.82 g, 1.79 mmol). The mixture was stirred under N_2 atmosphere for a period of 12 h. At the end of the reaction, the solvent was removed via a rotavap. To this residue was added hexane–dichloromethane (1:1, 20 mL) and filtered to remove further white solids. The filtrate was washed with aqueous sodium carbonate (5%) solution (10 mL). The organic phase was then dried and concentrated to give 1,14-pentaethylene-3,6,9,12-tetra(*n*-butylamine)-bis(methoxytriethyleneglycol ester malonamide) **5**, $(MEG_3)_2N_6C_4$, in 80% yield (1.32 g) as a yellow liquid. Spectroscopic data of the compound **5**: FT-IR (KBr) υ_{max} 3328 (w), 2952 (m), 2928 (m), 2862 (m), 2805 (m), 1736 (vs), 1667 (s), 1533 (m), 1456 (m), 1245 (m), 1104 (vs), 1032 (w), 938 (w), and 732 (m) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$, ppm) δ 4.31 (t, $J = 4.31$ Hz, 4H), 3.57–3.74 (m, 20H), 3.41 (s, 8H), 3.34 (m, 6H), 2.46–2.60 (m, 24H), 1.43 (m, 8H), 1.31 (m, 8H), and 0.93 (t, $J = 6.95$ Hz, 12H).

2.2.6 *Synthesis of 1,14-pentaethylene-3,6,9,12-tetra(n-butylamine)-bis(methoxytriethyleneglycol ester malonamide) quaternary ammonium salt 6, $(MEG_3)_2N_6^+C_4$*

A solution of 1,14-pentaethylene-3,6,9,12-tetra(n-butylamine)-bis(methoxytriethylene- glycol ester malonamide) **5** (0.50 g, 0.49 mmol) in anhydrous chloroform was added iodomethane (3.0 mL, excess, in portions) and stirred at 45°C for a period of 3.0 d. At the end of quaternization, the solvent was evaporated to afford the quaternary ammonium salt of 1,14-pentaethylene-3,6,9,12-tetra(*n*-butylamine)-bis(methoxytriethyleneglycol ester malonamide) **6**, $(MEG_3)_2N_6^+C_4$, Spectroscopic data of the methyl iodide salt **6**: FT-IR (KBr) υ_{max} 3318 (w), 2960 (m), 2929 (m), 2869 (w), 2851 (w), 1735 (s), 1669 (m), 1537 (m), 1456 (m), 1244 (m), 1097 (s), 1028 (m), 918 (s), 852 (w), and 727 (vs) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, ppm) δ 4.73 (br, –CO-NH–, 2H), 4.32 (t, $J = 4.31$ Hz, ester –O-CH_2–, 4H), 3.30–3.90 (m, br, –N^+-CH_2–/–N^+-CH_3, 36H), 3.51–3.67 (m, ether –O-CH_2–, 16H), 3.47 (s, amide –N-CH_2–, 4H), 3.43 (s, α-H, 4H), 3.40 (s, –OCH_3, 6H), 1.93 (s, 3H), 1.65–1.92 (m, 5H), 1.27–1.49 (m, 10H), and 1.05-0.95 (m, 12H).

2.2.7 *Synthesis of 1,14-pentaethylene-3,6,9,12-tetra(n-butylamine)-bis(methoxytriethyleneglycol ester [60]fullerenyl malonamide) quaternary ammonium salt 7, $[C_{60}(>MEG_3)]_2N_6^+C_4$*

Finely divided [60]fullerene (1.0 g, 1.40 mmol) was taken into a round bottom flask and added anhydrous toluene (700 mL) under nitrogen. The solution was stirred for 12 h at ambient temperature to ensure complete dissolution of C_{60}. To the resulting purple-colored solution added carbon tetrabromide (0.17 g, 0.51 mmol) followed by a solution of 1,14-pentaethylene-3,6,9,12-tetra(*n*-butylamine)-bis(methoxytriethyleneglycol ester malonamide) quaternary ammonium salt **6** (0.35 g, 0.23 mmol) in anhydrous DMF (100 mL). The solution mixture was stirred for an additional 30 min of stirring and added slowly 1.8-diazabicyclo[5.4.0]-undec-7-ene (DBU, 0.15 g, 0.98 mmol) over a period of 15 min. The color of solution slowly turns into brown in a reaction period of 10 h. The solution was then concentrated on a rotavap to roughly 100 mL. Upon the addition of methanol to this concentrated solution, the crude product was precipitated as brown solids which were collected via centrifugation. Unreacted C_{60} in the crude solids was removed by repeated washings with toluene (5 × 100 mL) until light color or clear in the washing solution or filtrate. The remaining product of 1,14-pentaethylene-3,6,9,12-tetra(*n*-butylamine)-bis(methoxytriethyleneglycol ester [60]fullerenyl malonamide) quaternary ammonium salt **7**, $[C_{60}(>MEG_3)]_2N_6^+C_4$, as a brown compound was obtained in 50% yield (0.343 g, after recovered C_{60}). Spectroscopic data of the compound **7**; FT-IR (KBr) υ_{max} 3426 (w), 2960 (m), 2921 (m), 2869 (m), 2813 (w), 1735 (s), 1686 (s), 1565 (m), 1454 (s), 1382 (m), 1245 (m), 1098 (s), 1026 (s), 846 (w), 729 (m), 578 (m), and 526 (vs) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$, ppm) δ 4.42 (m, br, 4H), 3.35–4.05 (m, br, 54H), 1.63 (m, 4H), 1.20–1.50 (m, br, 12H), and 0.88–0.99 (m, br, 12H).

3 Results and Discussion

Synthesis of dumbbell-shaped [60]fullerene nanostructures requires the use of two unsymmetrical malonyl

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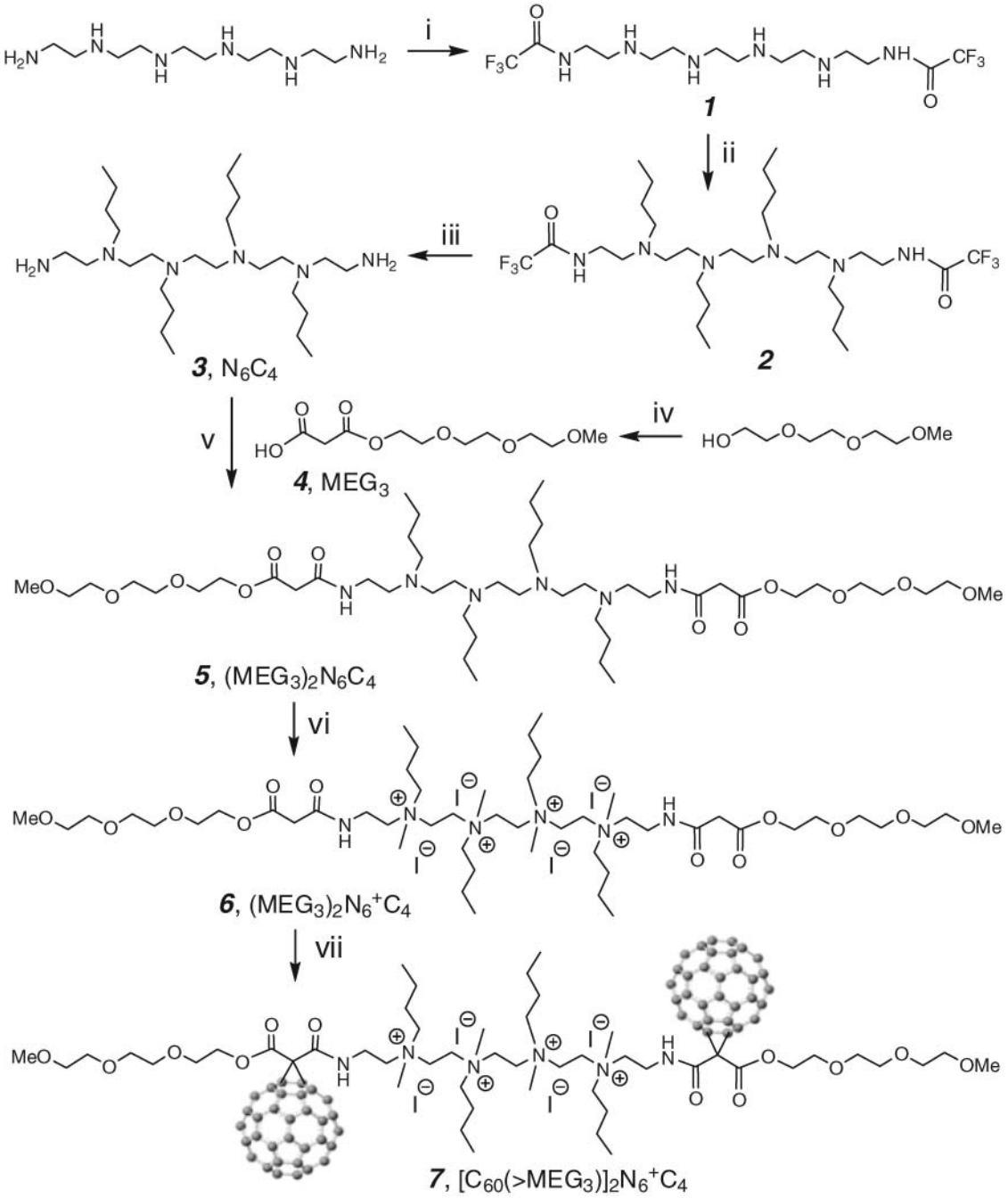

Sch. 1. Synthetic procedure, reagents, and conditions for the preparation of novel tetracationic dumbbell-shaped fullerene nanostructures $[C_{60}(>MEG_3)]_2N_6^+C_4$ **7**: i) ethyl trifluoroacetate, THF, 1.0 h; ii) $CH_3(CH_2)_2CHO$, $NaBH(OAc)_3$, CH_2Cl-CH_2Cl, r.t., 12 h; iii) K_2CO_3 (7%), MeOH–H_2O (7:3), r.t., 6.0 h; iv) meldrum acid, 95°C, 6.0 h; v) NHS, DCC, THF, r.t., 1.0 d; vi) Me–I, $CHCl_3$, 45°C, 3.0 d, and vii) C_{60}, DBU, toluene–DMF, r.t., 8.0 h.

derivatives as hinges interlinked by a common oligo(ethylene amine) subunit. Quaternization of each nitrogen atom of oligo(ethylene amine) chain, giving multiple quaternary ammonium salts, increases the solubility of C_{60} derivatives in water and polar solvents and charge-interactions with the anionic bio-substrate. To selectively control the alkylation of secondary amine moieties of oligo(ethylene amine) without causing the nucleophilic reaction of primary amine groups, a sequential protection-deprotection reaction pathway was applied, as shown in Scheme 1. The selection of *n*-butyl group moieties was aimed to balance the hydrophilicity and hydrophobicity characteristics of the molecule.

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Experimentally, primary amine groups of pentaethylene hexamine were selectively protected by acylation reaction with ethyl trifluoroacetate carried out at 0°C using the reported procedure (19). A nearly quantitatively yield (95%) of 1,16-di(trifluoroaceto)-pentaethylenehexamine **1** was obtained after removal of an excess amount of ethyl trifluoroacetate at elevated temperature. Reductive amination (20) reaction of the compound **1** was carried out first by capping all secondary amines with *n*-butyraldehyde forming the corresponding iminium salts at ambient temperature. It was followed by the reduction reaction of iminium salts using sodium triacetoxyborohydride as a reducing agent to afford pentaethylene-3,6,9,12-tetra(*n*-butylamine)-1,14-di(trifluoroacetamide) **2** in 95% yield. Subsequent removal of the protecting trifluoroacetyl groups of **2** took place in aqueous potassium carbonate (7%) in the presence of methanol (MeOH/H_2O ratio as 7:3) at room temperature for 6.0 h (21) to give 1,14-diamino pentaethylene-3,6,9,12-tetra(*n*-butylamine) **3** (N_6C_4) in a yield of 85%.

In a separated reaction, the intermediate of malonic acid methoxytriethyleneglycol ester **4** (MEG_3)was synthesized by directly heating meldrum's acid with monomethyl triethyleneglycol at 95°C for 6.0 h in neat condition to result in the product in 95% yield. Coupling reaction of **4** with tetra(*n*-butylamine)pentaethylene diamine **3** in the presence of *N*-hydroxysuccinamide (NHS) and *N*, *N*'-dicyclohexyl carbodiimide (DCC) in THF at ambient temperature for a period of 12 h afforded 1,14-pentaethylene-3,6,9,12-tetra(*n*-butylamine)-bis(methoxytriethyleneglycol ester malonamide) **5**, $(MEG_3)_2N_6C_4$,in 80% yield. Following quaternization of the compound **5** was performed using Me–I as the methylation agent at 45–50°C for a period of 3.0 d. A clean product 1,14-pentaethylene-3,6,9,12-tetra(n-butylamine)-bis(methoxytriethyleneglycol ester malonamide) quaternary ammonium salt **6**, $(MEG_3)_2N_6^+C_4$, as shown in Scheme 1, was obtained after thermal removal of the solvent and an excess amount of methyl iodide. Attachment of two C_{60} cages onto the quaternary ammonium salt of malonamide **6** was accomplished by the treatment of **6** in DMF with C_{60} pre-dissolved in toluene in the presence of 1.8-diazabicyclo[5.4.0]-undec-7-ene (DBU) at ambient temperature for a period of 10 h. In this reaction, carbon tetrabromide was applied as the bromination agent for the replacement of malonyl α-proton by the bromine atom *in situ*. To minimize the possible formation of partial products containing a monoaddition of C_{60}, an excess amount (6.0 equiv.) of [60]fullerene was applied. At the end of fullerenation, the residual C_{60} molecules were removed by repeatedly washing the crude product with toluene until the observation of a clear toluene solution in washings. The procedure led to 1,14-pentaethylene-3,6,9,12-tetra(*n*-butylamine)-bis(methoxytriethyleneglycol ester [60]fullerenyl malonamide) quaternary ammonium salt **7**, $[C_{60}(>MEG_3)]_2N_6^+C_4$in a yield of 50%.

All intermediates and products **2**–**7** were characterized structurally by using various spectroscopic techniques.

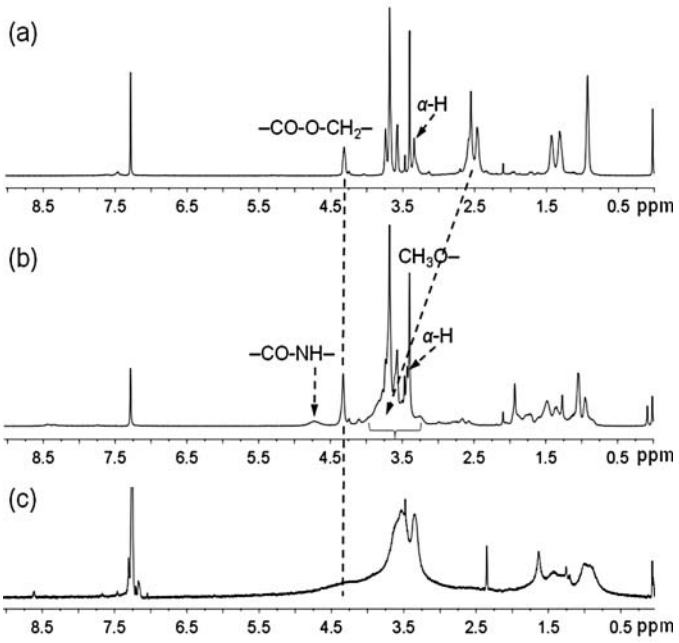

Fig. 1. ^{1}H-NMR spectra of (a) malonamide $(MEG_3)_2N_6C_4$ **5**, (b) quaternary ammonium salt of malonamide $(MEG_3)_2N_6^+C_4$ **6**, and (c) quaternary ammonium salt of [60]fullerenyl malonamide $[C_{60}(>MEG_3)]_2N_6^+C_4$ **7** in $CDCl_3$.

Successful bis-acylation of pentaethylene hexamine to the formation of the compound **1** was clearly observed in its ^{1}H NMR spectrum where the chemical shift of α-protons (4H) as trifluoroacetamide *N*-attached methylene protons was detected at δ 3.41 in nearly 1.0 ppm down field shift from that of the precursor compound. The FT-IR spectrum of **1** also exhibits a new strong absorption band at 1704 cm^{-1} corresponding to an amide carbonyl stretching. In the reductive amination step of **1** to the formation of the *n*-butylated compound **2**, integration ratios of alkyl and alkylamino protons in the ^{1}H-NMR spectrum of **2** match well with four additional butyl groups, consistent with four secondary amines of **2**. We assigned all proton peaks at δ 3.38–3.43 (6H), 2.40–2.70 (24H), 1.30–1.40 (16H), and 0.92 (12H) to amide *N*-attached $–C_\alpha H_2–$ (4H) and amide –NH– (2H), all amine *N*-attached $–C_\alpha H_2–$ of *n*-butyl groups and ethyleneamino moiety, $–C_\beta H_2–/–C_\gamma H_2–$, and $–CH_3$ end groups of *n*-butyl moiety, respectively. Upon deprotection of trifluoroacetamide of **2**, the compound **3** (N_6C_4) showed disappearance of the amide carbonyl band at 1705 cm^{-1} along with all proton peaks in connection with the amide moiety at δ 3.38–3.43 which shifted up-field to δ 2.72 (m, 4H).

In the case of the triethyleneglycolated malonic acid **4**, its ^{1}H-NMR spectrum displayed peaks at δ 10.22 (1H), 4.31 (2H), 3.60–3.75 (10H), and 3.36–3.42 (5H) indicating clearly the presence acid proton, ester-linked –O-CH_2– protons, all ethylene ether –O-CH_2– protons, and –O-CH_3 merged with two malonyl α-protons, respectively.

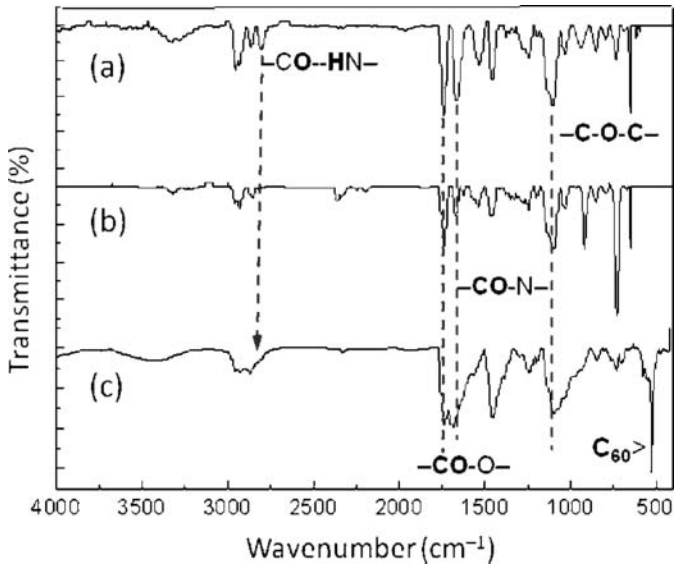

Fig. 2. Infrared spectra of (a) malonamide $(MEG_3)_2N_6C_4$ **5**, (b) quaternary ammonium salt of malonamide $(MEG_3)_2N_6^+C_4$ **6**, and (c) quaternary ammonium salt of [60]fullerenyl malonamide $[C_{60}(>MEG_3)]_2N_6^+C_4$ **7** collected on a KBr pellet.

Bis-amidation conversion of **3** and **4** to the key intermediate bis(triethyleneglycol ester malonamide) **5** [$(MEG_3)_2N_6C_4$] led to the appearance of new amide –NH– (4H) proton peaks at δ 3.34 which was down-field shifted from that of **3** at δ 2.72, as shown in Figure 1a, indicating the successful functional transformation. The remaining proton peaks at δ 4.31 (4H), 3.57–3.74 (20H), 3.41 (8H), 3.34 (6H), and 2.46–2.60 (24H) were assigned to the chemical shift of ester-linked –O-CH_2– protons, all ethylene ether –O-CH_2– protons, –O-CH_3 merged with two malonyl α-protons, amide –N-CH_2– merged with another two malonyl α-protons, and all amine *N*-attached –$C_\alpha H_2$– of *n*-butyl groups and ethyleneamino moiety, respectively (Figure 1a). In addition, both ester and amide carbonyl stretching IR absorptions at 1736 and 1667 cm^{-1}, respectively, as shown in Figure 2a, were detected along with a strong band at 1104 cm^{-1} corresponding to the absorption of glycol ether –C-O-C– moiety. Interestingly, an intermolecular hydrogen bonding band arising from amide –NH– absorption at 2805 cm^{-1} was observed that is consistent with the structure of **5**.

The most significant spectroscopic feature on quaternization of the compound **5** leading to the formation of tetramethyl malonamide quaternary ammonium salt **6** $(MEG_3)_2N_6^+C_4$ was the large down-fielded shift (more than 1.0 ppm) of all amine *N*-attached –$C_\alpha H_2$– protons of ethyleneamino moiety at δ 2.46–2.60 to δ 3.30–3.90 (36H) for quaternary ammonium methylene protons (–N^+-CH_2–) and methyl protons (–N^+-CH_3) as a broad band (Fig. 1b). In this chemical shift region, sharp peaks distributed at δ 3.51–3.67 (16H), 3.47 (4H), 3.43 (4H), and 3.40 (6H) were assigned to the chemical shift of ether –O-CH_2–, amide –N-CH_2–, α-H, and –OCH_3 protons, respectively, with a slight down-field shift depending on the distance away from the quaternary ammonium moiety. The chemical shift of ester –O-CH_2– proton remains nearly identical at δ 4.32. Interestingly, the amide –CO-NH– proton is detectable at δ 4.73 as a broad band clearly indicating no methylation on the amide nitrogen atom under the current reaction conditions.

Attachment of two C_{60} cages onto the key intermediate of quaternary ammonium salt **6** at the malonamide moieties giving the final product quaternary ammonium salt of [60]fullerenyl malonamide **7**, $[C_{60}(>MEG_3)]_2N_6^+C_4$, was verified by the observation of a sharp fullerenyl cage absorption band at 526 (vs) and 578 (w) cm^{-1} in the infrared spectrum of **7**, as shown in Figure 2c. It was accompanied with several main characteristic IR absorption bands including the ester and amide carbonyl stretching at 1736 (vs) and 1686 (vs) cm^{-1}, respectively, glycol ether –C-O-C– stretching at 1104 (vs) cm^{-1}, and intermolecular hydrogen bonding of amide –CO-NH– at 2814 cm^{-1}, consistent with the combination of structural groups of **6** and C_{60} cages. Furthermore, ^{1}H-NMR of **7** showed the chemical shift value retention of nearly all protons from those of **6** except significant peak broadening, perhaps, due to the fullerene cage effect that restricts sterically the degree of rotational freedom along the bonds in close vicinity of C_{60}. UV-Vis spectrum of the compound **7** (Fig. 3c) displayed two main absorption bands at 257 and 325 nm which matched well with the characteristic bands of C_{60} cage as the functionalized monoadduct.

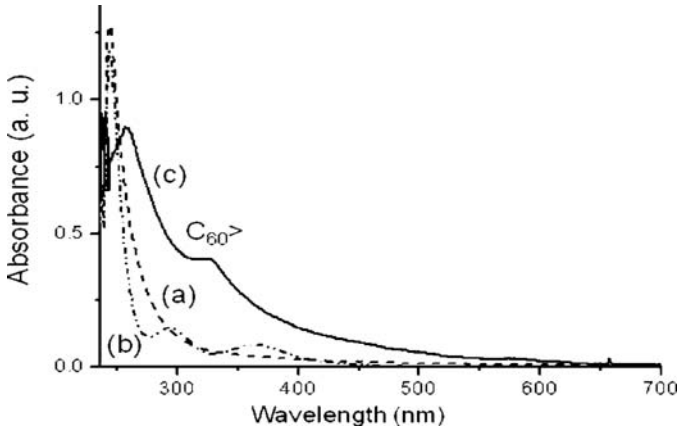

Fig. 3. UV-Vis spectra of (a) malonamide $(MEG_3)_2N_6C_4$ **5**, (b) quaternary ammonium salt of malonamide $(MEG_3)_2N_6^+C_4$ **6**, and (c) quaternary ammonium salt of [60]fullerenyl malonamide $[C_{60}(>MEG_3)]_2N_6^+C_4$ **7** in $CHCl_3$ at a concentration of 1.0×10^{-5} M.

4 Conclusions

A design of novel hydrophilic tetracationic dumbbell-shaped [60]fullerene nanostructures was made for enhancing solubility in both water and polar bio-media and good

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compatibility in hydrophobic bio-media for the potential application of the materials as photodynamic sensitizers in the PDT treatment. Selection of alkyl moieties was aimed to balance the hydrophilicity and hydrophobicity characteristics of the fullerene derivative. A sequential protection-deprotection reaction pathway was applied for the functional differentiation between primary and secondary amine moieties of pentaethylene hexamine. Synthesis of the target molecule involves two key steps of unsymmetrical esterification and amidation of malonic acid and subsequent fullerenation. Interestingly, the attachment of highly hydrophobic C_{60} cage to an incompatible, highly water-soluble malonyl intermediate containing oligo(ethylene oxide) and quaternary ammonium salts can be accomplished without difficulty by the proper selection of the alkyl chain length. The synthetic strategy was achieved using mild reaction conditions in the intermediate molecule preparation and led to a moderate overall product yield.

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